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chlorophyll spectrum in a single leaf, but all leaves give all these bands when two or three are used. The bands in the spectrum of a solution of chlorophyll were compared with those in the living leaf in two ways: (1) by observing the two spectra at the same time, one with direct light and the other by means of the comparison prism; (2) by observing the position of the bands in direct sunlight with reference to the Fraunhofer lines. In all cases the bands appear in exactly the same place in the spectrum of a leaf as in that of a standard solution giving the normal chlorophyll spectrum.

From the above it is plain that the most typical spectra are yielded by those non-acid leaves from which the chlorophyll can be extracted most easily, namely (in the order of their excellence) *Primula obconica*, *Raphanus sativus*, *Vicia Faba*, *Abutilon* (young leaf), *Avena sativa*, *Cestrum elegans* (young leaf), *Euphorbia pulcherrima* (young leaf), *Lycopersicum esculentum*, *Primula sinensis*, and *Ricinus communis*.

Another well-known optical property of chlorophyll is its fluorescence, all solutions of chlorophyll being slightly fluorescent. The products of the decomposition of chlorophyll, however, seem to be more fluorescent than chlorophyll itself. A solution of chlorophyll extracted with boiling alcohol from boiled leaves in the light is more fluorescent than a solution of unchanged chlorophyll. Of course some leaves are better than others, and of all the leaves in the above table, the very best are *Jacobinia magnifica*, *Cineraria cruenta* (the dark-leaved varieties), *Cestrum elegans*, and *Hedera Helix*. Standard solutions from these leaves give a brilliant blood-red fluorescence.—SOPHIA ECKERSON, *Smith College, Northampton, Mass.*

FURTHER OBSERVATIONS ON THE STRUCTURE OF THE STARCH GRAIN.¹

IN a previous paper (1) on the structure of the starch grain, I showed that the peripheral layer of the potato starch grain breaks and recurves on treatment with certain reagents, much like the cutin layer of an epidermal cell on treatment with sulfuric acid. While I had previously observed that this peripheral layer is stained with certain of the aniline dyes, I thought that the effect might probably be due to the remains of plastids or protoplasmic material, rather than to the presence of a distinct membrane. I am now satisfied, however, that it can be demonstrated that the peripheral layer of the starch grain is a distinct membrane.

It is well known that upon the addition of an iodine solution to unaltered

¹ Read at the meeting of the Society for Plant Morphology and Physiology, December 1903.

starch grains at the ordinary temperature, the grains are colored blue; while the solution or filtrate remains colorless or some shade of yellow, depending upon the amount or strength of iodine solution that has been used. If on the other hand the grains are triturated with sand (one part of starch to five of sand) for a short time previous to the addition of the water, the filtrate will be colored a deep blue upon the addition of an iodine solution. Heretofore it has been supposed by some that the blue coloration was due to the presence of fine particles which went through the filter and were suspended in the filtrate, while in reality there is a true solution of the starch; or in other words the soluble starch has been liberated from the grain by the rupture of the peripheral layer. A polariscopic examination of the starch solutions, after clarification by means of talcum, showed that they have marked dextro-rotatory properties. The angle of rotation for solutions of corn, wheat, maranta, and potato starch ranged from 0.3814 to 0.8770° . Furthermore, a microscopic examination of the triturated starch shows the grains to be in various stages of disintegration, and this taken together with the behavior of starch toward iodine, as well as the other observations referred to, would indicate that the starch grain consists of a membrane that is insoluble in water at ordinary temperature; and an interior portion which is at least in part soluble in water at ordinary temperature, that is consists of soluble starch or starch that is colored blue with iodine. This technique enables us to differentiate at once the soluble starch present, which heretofore has not been supposed to exist in the unaltered starch grain. As to whether this substance is identical with the granulose of NÄGELI (2) is a problem which remains to be determined. It may be added that this method furnishes a means of separating the constituents in the unaltered grain, and is to be preferred to the methods which have been employed heretofore, of using solutions of ferments or mineral acids, which give rise to altered substances, and hence do not give a true differentiation of the substances as they naturally occur in the starch grain.

This brings us then to consider the views of RASPAIL (3), in connection with those of others, in regard to the nature of the starch grain, which may be summarized as follows:

1. The starch grain consists of a membrane which is insoluble in water, and a more or less soluble content, as pointed out by RASPAIL.
2. It develops from a centric or excentric point, to which layer after layer is added, a view first advanced by FRITSCH (4), and subsequently enlarged upon by SCHIMPER (5), who demonstrated that its growth is dependent upon the function of the leucoplastids.

3. The content of the grain consists of at least two different substances, as first pointed out by NÄGELI (2), and later confirmed by MEYER (6), SCHIMPER (5), and others, who showed in addition that the structure might be compared to that of sphero-crystalloids.

THE ACTION OF IODIN UPON STARCH.

The effect of iodine upon starch has been the subject of considerable investigation and speculation by both botanists and chemists. MYLIUS (7) held to the view that the blue coloration formed on the addition of iodine to starch is due to the formation of a compound of hydriodic acid and starch. MEINEKE (8) also contended that hydriodic acid is essential to the formation of the blue color of an iodine-starch solution. STOCKS (9), SEYFERT (10), and ROUVIER (11) are of the opinion, however, that this colored compound does not contain hydriodic acid, the last named investigator having shown that it may be formed in the presence of alcohol as well as water, and all of them agreeing that a true chemical compound (iodide of starch) is formed.

On the other hand, KÜSTER (12) and MEYER (6) believe that the so-called iodide of starch is not a true chemical compound, but that the iodine and starch are combined in much the same way as the ingredients in an emulsion. This latter view has been more or less sanctioned on account of the statements in the textbooks that the iodine is removed from the combination on treatment with carbon disulfide. My observations, however, show that if the starch is in excess of the iodine (using 1 gm of potato starch and 10 cc of a solution containing 0.1 per cent. of iodine and 0.5 per cent. of potassium iodide), or if the iodine and starch be in such proportion that all of the free iodine is taken up, and the mixture is dried before adding the carbon disulfide, even on allowing it to act for six months, the carbon disulfide is not colored; that is, does not take up the iodine, the same being true of alcohol and chloroform. This result, taken together with the previous experiments in the treatment of starch with sand and then with iodine, would seem to confirm the view that a true chemical compound is produced by iodine and soluble starch, whether in the grain or in solution.

That the amount of soluble starch varies in the starch grains of different origin can be demonstrated by the treatment of definite amounts of the starches with definite quantities of iodine solution. On treating 0.5 gm of the several commercial starches with 2 cc of iodine solution (0.1 per cent. of iodine and 0.5 per cent. of potassium iodide), it will be found that there is a marked difference in the intensity of the color in the grains, those of potato and maranta being colored deep blue, while those of corn and wheat

are scarcely at all colored, the mixtures being somewhat of a purplish color. These observations seem to accord with the experiments of ROUVIER (13), who found that the different classes of starch took up varying amounts of iodine, and are contrary to the statement by STOCKS (9) that the shade of color varies with the amount of iodine but not with the different starches. In other words, the shade and intensity of color not only vary according to the strength of iodine solution, but also according to the kind of starch used.

It is well known that if a starch solution be treated with iodine in the cold, a blue coloration will be the result, and that if this solution be then heated the blue color will disappear, but will return on cooling the solution, though less intensely than before. This peculiar behavior of starch and iodine has never been satisfactorily explained. NÄGELI and SCHWENDENER (14) consider that the loss of color on the application of heat is due to the production of hydriodic acid; but this does not explain the reappearance of the color on cooling; and so far as I am aware no one has ever demonstrated the presence of this acid. If this acid were present, then according to MYLIUS (7) and MEINEKE (8), the blue color would remain on heating the solution, since they claim, as already pointed out, that the blue color is due to the presence of this acid. It is thus apparent that there is considerable contradiction in the arguments that have been presented on this subject.

The following experiments tend to explain this phenomenon. If we take 0.5^{gm} of potato starch and mix it with 50^{cc} of water and heat the mixture in an Erlenmeyer flask of about 100^{cc} capacity on a water bath to a temperature of about 80° C. for one hour, and then add 5^{cc} of iodine solution (iodine 0.1 per cent. and potassium iodide 0.5 per cent.) and place a piece of ordinary filter paper, which usually contains starch, over the top of the flask, and then continue the heating until the solution is decolorized, which takes place at about 80° C., the filter paper will be colored blue, showing that some of the iodine has been volatilized. Furthermore, on cooling the solution, as already pointed out, the blue color begins to return at about 65° C. and increases in intensity until at about 40° C. it reaches the maximum; though it is less intense than the color produced when an iodine solution is added to a starch solution cooled to this temperature. These results may be interpreted as showing that iodine forms a compound with starch which is dissociated on the application of heat; that is, at a temperature between 60 and 80° C., part of the freed iodine is volatilized as such, and the amount remaining in solution depends upon the length of time the heat is applied. Also the loss of iodine in this way accounts for the decrease in color of the starch-iodine solution on cooling.

An attempt was made to remove the free iodine from the hot colorless starch-iodine solution by the addition of chloroform, but it was found difficult to handle the material, as chloroform boils at about 60° C. Subsequent experiments, however, showed that unaltered potato starch grains as well as a solution of potato starch would remove the iodine from a chloroformic solution at the ordinary temperature. These experiments were conducted as follows: 5^{cc} of an iodine solution, of the strength already stated, were shaken in a separatory funnel with 25^{cc} of chloroform, and most of the chloroform containing the iodine separated. To this was added 5^{gm} of dried starch. The latter, however, did not take up the iodine from the solution; but on the addition of a small quantity of water (about 10^{cc}) and after shaking the mixture, the starch and iodine combined, and almost all of the iodine in the chloroformic solution may be removed in this way. The addition of 2^{cc} of potassium iodide solution (5 per cent.), even with 0.5^{gm} of starch, causes the iodine to be taken up immediately. The starch in a soluble starch solution also combines immediately with the iodine in a chloroformic solution.

In summing up the observations on the behavior of iodine and starch, it seems to me that we are dealing with a chemical compound of iodine and soluble starch; but that the combination is a feeble one, being easily dissociated upon the application of heat, and the iodine being more or less volatilized. Also the facility with which soluble starch takes up the iodine in a chloroformic solution indicates that the affinity of starch for iodine is considerably greater than heretofore supposed.

STAINING OF THE GRAIN.

While I have obtained at times some beautiful results by the use of aniline stains, it has been impossible for me until now to duplicate some of my earlier results. I have succeeded, however, in developing a method in the staining of wheat starch which yields uniformly satisfactory results. It is as follows: to 0.500^{gm} of wheat starch add 2^{cc} of an aqueous iodine solution (containing 0.1 per cent. of iodine and 0.5 per cent. of potassium iodide); mix well and allow the mixture to stand from 20 to 30 minutes in a porcelain dish or watch crystal; then add 2^{cc} of a saturated aqueous solution of gentian-violet (1^{gm} of gentian violet to 100^{cc} of water); allow this to stand from 12 to 24 hours, examining the grains from time to time by mounting them in water. When the grains are satisfactorily stained, the mixture is transferred to a filter and the excess of stain is removed as quickly as possible by washing the magma with water. The material is then allowed to dry spontaneously or between pieces of bibulous paper.

For examination it is then mounted in Canada balsam, the preparation being permanent for years, as is also the case with the unmounted material.

Corn starch may also be stained by the use of this method, but in the staining of potato and maranta starches it is necessary to use weaker iodine solutions. I have not evolved entirely satisfactory methods, however, for uniformly staining these latter starches.

It may be of interest to state that the foregoing method has certain features which are similar to those of the gentian-violet method used in demonstrating the so-called continuity of protoplasm in the vegetable cell wall (15). In the study of the continuity of protoplasm a swelling reagent, such as sulfuric acid, is used and a comparatively short time is consumed in the whole operation; whereas in the method proposed for the staining of wheat starch the water used may be considered a swelling agent acting on the grain during a longer time. The analogy in the results are so striking that students who are interested in the study of the continuity of protoplasm will do well to compare their results on the cell wall with those obtained in the study of the wheat starch grain by the method just described.

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